10 February 2015
EMA/CVMP/769137/2014
Committee for Medicinal Products for Veterinary Use (CVMP)

European public MRL assessment report (EPMAR)

Lasalocid (modification of the ADI and MRLs in poultry)

On 1 December 2014 the European Commission adopted a Regulation[1] modifying the maximum residue limits for lasalocid in poultry, valid throughout the European Union. This modification was based on the favourable opinion and the assessment report adopted by the CVMP.

Lasalocid is used in bovine species for the treatment of coccidiosis and in poultry for the prevention of coccidiosis caused by *Eimeria spp.*

Maximum residue limits had previously been established for lasalocid in bovine species and poultry. Pfizer Animal Health submitted to the European Medicines Agency the application for the modification of ADI and maximum residue limits in poultry, on 23 October 2012.

Based on the original and complementary data in the dossier, the CVMP recommended on 12 December 2013 the modification of ADI and maximum residue limits for lasalocid in poultry.

Subsequently the Commission recommended on 10 September 2014 that maximum residue limits in poultry are established. This recommendation was confirmed on 20 October 2014 by the Standing Committee on Veterinary Medicinal Products and adopted by the European Commission on 1 December 2014.

[1] Commission Implementing Regulation (EU) No 1277/2014, O.J. L346, of 2 December 2014

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Summary of the scientific discussion for the establishment of MRLs

Substance name:	Lasalocid
Therapeutic class:	Anti-infectious agents/Antibiotics
Procedure number:	EU/12/204/PFZ
Applicant:	Pfizer Animal Health
Target species:	Poultry
Intended therapeutic indication:	Coccidiostat
Route(s) of administration:	Oral by feed

1. Introduction

Lasalocid is an antibiotic from the group of carboxylic ionophores and is used as the sodium salt (CAS No 25999-20-6). Lasalocid is produced by *Streptomyces lasaliensis* and is a mixture of several closely related homologue substances A, B, C, D, and E. The sum of the lasalocid homologues B, C, D, and E is limited to 10% of the total weight of the active substance, lasalocid. The substance is mainly active against gram-positive microorganisms.

In veterinary medicine lasalocid is used in poultry for the prevention of coccidiosis caused by *Eimeria spp* administered in feed at doses of 75 to 125 mg/kg feed in chickens and turkeys and 7 to 14 mg/ kg bw per day in-feed from day old to a maximum of 12 weeks for pheasants and partridges.

In cattle the substance is used for the treatment of coccidiosis in young (non-lactating) cattle.

Lasalocid is authorised as a feed additive under Regulation (EC) No 1831/2003 for the prevention of coccidiosis in chickens and turkeys. As a feed additive the substance is given continuously to chickens and turkeys from day 0 up to 16 weeks, at doses of 75 to 125 mg/kg in feed with a withdrawal period of 5 days.

Lasalocid is not used in human medicine.

The CVMP has previously assessed the consumer safety of lasalocid sodium and established an ADI of 2.5 µg/kg/day (i.e. 150 µg/60 kg bw person/day).

Currently lasalocid is included in Commission Regulation (EU) No 37/2010 of 22 December 2009 (Commission Implementing Regulation (EU) No 86/2012) in accordance with the following table:

Pharmaco-logically active substance	Marker residue	Animal species	MRLs	Target tissues	Other provisions	Therapeutic classification
Lasalocid	Lasalocid A	Poultry	20 µg/kg 100 µg/kg 100 µg/kg 50 µg/kg 150 µg/kg	Muscle Skin and fat Liver Kidney Eggs	NO ENTRY	Anti-infectious agents/ Antibiotics
		Bovine	10 µg/kg 20 µg/kg 100 µg/kg 20 µg/kg	Muscle Fat Liver Kidney	Not for use in animals from which milk is produced for human consumption	

Pfizer Animal Health submitted the application for the modification of the ADI and the maximum residue limits in poultry to the European Medicines Agency, on 23 October 2012 based on the differences between the safety evaluation carried out by the European Food Safety Authority (EFSA) related to the assessment of lasalocid as a feed additive and by the CVMP with regard to the assessment as a medicinal product.

2. Scientific risk assessment

New interpretation of toxicity data with regard to neurological effects has been presented in this application with the intention to align the ADI with that established by EFSA and subsquently to increase the MRLs for poultry.

Three opinions of EFSA in relation to use of a lasalocid containing product as a feed additive in a number of avian species were submitted as the basis for the request to modify the toxicological ADI for lasalocid. New data concerning tissue residues and analytical methods for chicken and other minor species were submitted.

2.1. Safety assessment

Lasalocid sodium was previously assessed by the CVMP and a toxicological ADI of 2.5 µg/kg bw, i.e. 150 µg/person was established based on the NOEL of 0.5 mg/kg/day from the 2-year chronic oral toxicity study in rats and the maternal toxicity study in rabbits and applying an uncertainty factor of 200 due to the limited data in respect of neurotoxicity.

A microbiological ADI was also calculated by the CVMP as 4.91 µg/kg bw i.e. 294.6 µg/60 kg person.

Lasalocid was assessed by EFSA with regard to the use of the substance as a feed additive. The FEEDAP Panel of the EFSA also considered the NOEL of 0.5 mg/kg/day from the 2-year chronic oral toxicity study in rats and the maternal toxicity study in rabbits as the most relevant end point to derive the ADI. However in calculating the ADI the FEEDAP Panel of the EFSA applied an uncertainty factor of 100 which resulted in a higher ADI (5 µg/kg bw, i.e. 300 µg/person) than the one established by the CVMP.

Considering that the toxicological ADI is lower than the microbiological ADI, the toxicological ADI of 2.5 µg/kg/day (150 µg/person/day) was considered the overall ADI for the assessment of the safety of the consumer.

For the assessment of the request to modify the ADI the CVMP considered relevant safety data previously assessed, in particular regarding neurological effects, the EFSA opinion and microbiological effects.

2.1.1. Overview of pharmacological properties

No new pharmacological data were submitted; the evaluation previously carried out by the CVMP is summarised here below.

Pharmacodynamic properties including mode of action

Lasalocid sodium is a carboxylic acid ionophore that binds divalent and monovalent cations. The alteration of ionic transport across lipid membranes evokes catecholamine release from cells. A positive ionotropic effect was observed in dogs following intravenous administration of lasalocid at 1 mg/kg bw and increased coronary and renal blood flow was also demonstrated. *In vitro* data demonstrates reversible effects upon Golgi apparatus in mammalian cellular preparations at 10 µg/ml (incubate) and increased serotonin secretion from platelets at 0.2 µM. No further data on pharmacodynamics were available.

Pharmacokinetic properties (mainly in laboratory animals)

Pharmacokinetics after a single administration of 1 mg/kg bw ^{14}C-lasalocid orally to mice and rats indicate a rapid absorption of lasalocid. Peak blood concentrations of 0.7 µg/ml and 0.05 µg/ml were obtained at 0.25 hours and 3 hours in mice and rats, respectively. The blood elimination half-life was 3 and 4.8 hours for mice and rats, respectively. In both species nearly 90 to 95% of the radioactivity administered was recovered in faeces 48 hours post-administration, indicating an almost complete faecal elimination of lasalocid residues. Lasalocid shows a widespread distribution in rats and mice, affecting many tissues such as muscle, liver, skin, fat, heart, thymus, lung, spleen, etc. The tissue with highest concentrations was the liver with peak concentrations ranging from 2500 to 4000 µg/kg. The urinary excretion of radioactive residues was nearly 1% in both species. After administration of 1 mg/kg bw of ^{14}C-lasalocid, by oral gavage, to biliary duct cannulated rats, 60% of the dose was absorbed from the gastrointestinal tract to the blood. Fifty eight percent of the administered dose was recovered in bile, indicating that nearly 100% of the absorbed dose was excreted by the biliary duct. Only 1.1% of the administered dose was recovered in urine.

2.1.2. Calculation of pharmacological ADI, if relevant

Not relevant.

2.1.3. Overview of toxicology

No new toxicological data were submitted, however a new interpretation of the neurological effects was provided; the evaluation previously carried out by the CVMP is summarised here below.

Single-dose toxicity

The acute toxicity of lasalocid has been investigated following oral, dermal, intraperitoneal, and subcutaneous routes of exposure. By oral route the toxicity of lasalocid sodium was assayed in mice, rats, neonatal rats, and rabbits. In these animal species lasalocid showed moderate, acute oral toxicity in mice and adult rats, with oral LD_{50} values of 146 and 122 mg/kg bw, respectively. Lasalocid was highly toxic by oral route in neonatal rats and rabbits, with oral LD_{50} values of 33 and 40 mg/kg bw, respectively. The acute dermal toxicity was assayed in rabbits. Following 24 hours exposure, animals were observed for a further 14 days and lasalocid showed a low acute toxicity with an approximate dermal LD_{50} of 1400 mg/kg bw calculated. The acute intraperitoneal toxicity was investigated in mice and rats. Signs of toxicity included tremors in mice and cyanosis, decreased motor activity, and respiratory depression in rats. Dose-related increases in mortality were observed in both species; LD_{50} values were 68 and 26.5 mg/kg bw for mice and rats, respectively. Acute toxicity *via* the subcutaneous route was investigated in one mice study and the LD_{50} was calculated to be 140 mg/kg bw. Rabbits and neonatal rats indicated an increased susceptibility to lasalocid.

The acute oral toxicity of lasalocid in chickens has been investigated in two single-dose studies. In the first single-dose study, lasalocid sodium was orally administered to broiler-type chickens, *via* capsules, at different doses from 39 to 317 mg/kg bw. Toxicity onset was rapid and clinical signs included lethargy, wing droop, resting on hocks, and death generally within 24 hours. Birds dying later showed emaciation and dehydration. Nephromegaly, splenomegaly, and hepatomegaly with scattered foci of necrosis were seen at necropsy. LD_{50} values of 59 and 84 mg/kg bw were calculated for each batch. In the second acute study, birds were gavage dosed with lasalocid sodium, either using 5% acacia gum or 5% of an emulsion product as the vehicle. Oral LD_{50} values of 112 and 84 mg/kg bw, respectively, were calculated for lasalocid sodium in each of the two vehicles. A dose-related decrease in body weight was observed with each vehicle. A higher toxicity of lasalocid in horses and young animals such as calves up to 7 days of age was observed. The estimated oral LD_{50} of lasalocid in horses is 21.5 mg/kg bw. The clinical syndrome includes depression, ataxia, paresis, paralysis, anorexia and recumbency. Cardiac muscle is strongly affected. Doses of 5 to 8 mg/kg bw have caused lethal effects in young calves (single or multiple doses).

Repeat-dose toxicity

Three 13-week oral toxicity studies were performed in CD rats. These studies used adult rats, weanling rats, and weanlings derived from treated parents. The observed effects on biological parameters were generally consistent across the three rat studies and female animals showed a consistently greater sensitivity to the effects of lasalocid administration than male rats. Common findings included decreased haematocrit and haemoglobin, leucocytosis, small numbers of target cells, a resistance of erythrocytes to lyses by osmotic stress, increased haemosiderin levels in the liver and kidneys, elevated alkaline phosphatase (ALP), aspartate aminotransferase (AST) and alanin aminotransferase (ALT), and vacuolation of cardiac muscle.

In the 13-week oral toxicity study in weanling rats, lasalocid sodium was administered in the diet at doses of 1, 2, 3 and 10 mg/kg bw/day. Neurological examinations, consisting of observations of gait, body position, muscle tone, movement of the legs, and reflexes; ophthalmoscopic examinations, haematology, serum chemistry and urinalysis measurements were performed. At the end of the treatment, all surviving animals were necropsied and examined for gross abnormalities. Reduced bodyweight gain and/or decreased bodyweight, was seen at 10 mg/kg bw, and was more significant in females. Haematological effects, including decreased haematocrit, decreased haemaglobin levels, small numbers of target cell erythrocytes, and slight neutrophilic leucocytosis, were observed in treated animals, but were more extensive in females – haematological effects were seen in females dosed at 2

mg/kg bw and above and in males dosed at 3 mg/kg bw and above. A decrease in the susceptibility of erythrocyte membranes to osmotic stress was seen in some 10 mg/kg bw dosed animals. Biologically significant increases in serum alkaline phosphatase (ALP) and elevated aspartate aminotransferase (AST) levels were seen in females receiving 10 mg/kg bw. Altered serum electrolyte values were also seen at 10 mg/kg bw. Vacuoles in the cardiac muscle were observed in females treated with 10 mg/kg bw and increased levels of haemosiderin in the kidneys of females and the liver of both sexes, but more apparent in females, at doses of 3 and 10 mg/kg bw. A NOEL of 1 mg/kg bw/day was established, based on the occurrence of a slight decrease in haematocrit and a slight neutrophilic leucocytosis in females at 2 mg/kg bw/day. A 13-week oral toxicity study was performed in Beagle Dogs (doses: 2, 5 and 10 mg/kg bw/day *via* capsules). General behaviour, signs of toxicity and food consumption were observed daily, and body weights were recorded once per week. Ophthalmoscopic, neurological and electrocardiogram examinations, and haematology and serum chemistry measurements, were performed prior to treatment and at approximately monthly intervals. At the end of the treatment, all dogs were necropsied and examined for gross abnormalities and histology. A slight, but statistically significant decrease in serum chloride values was observed for dogs treated with 5 mg/kg bw and 10 mg/kg bw lasalocid sodium compared to concurrent control. This decrease may be biologically meaningful. Although neurological clinical signs (muscular weakness and/or tremors of the hind limbs, awkward gait) were observed, no histopathological changes were noted in the central nervous system and peripheral nerves of treated dogs. Vacuolation of hepatic parenchymal cells was observed in three females treated with 10 mg/kg bw. Hydropic cellular changes are generally reversible and are non-specific. From the results of this study a NOEL dose of 2 mg lasalocid/kg bw/day can be retained.

A 2-year oral chronic toxicity study was performed in Beagle dogs (male and female). Lasalocid was administered with the feed at 10, 35, 180 mg/kg feed. Clinical signs and food consumption were measured and recorded daily. Body weights were measured weekly. Physical and neurological examinations and palpation for tissue masses were performed pre-test and at monthly intervals. The neurological examination included the observation of the following reflexes: righting, patellar, flexor, extensor, visual placing response, corneal and pupillary. Central nervous system observations included: behaviour, movements, reactivity to stimuli and muscle tone. Opthalmoscopic examinations, electrocardiograms and haematology, clinical chemistry and urinalysis parameters were measured. At the end of the treatment, all surviving dogs were necropsied and examined for gross abnormalities and histology. A slight decrease in food consumption was seen in high-dose animals during the first three months of treatment. Elevated levels of alkaline phosphatase (ALP) were seen in high-dose animals from month 6 to study termination. Neurological clinical signs were observed (intermittent paralysis of the limbs) in high-dose animals. Nevertheless, no histopathological changes were noted in the central nervous system and sciatic nerves of dosed dogs. Significant reduction in the prostate weight was seen in high-dose males. In opthalmoscopic examinations, retinal lesions were observed, but the lesions may have had an inflammatory basis. Changes in organ weights were not associated with histopathological lesions. A NOEL of 35 mg lasalocid/kg feed (equivalent to approximately 1 mg/kg bw/day in both sexes) was established, based on the occurrence of transiently reduced food consumption and persistently elevated serum alkaline phosphatase (ALP) activity, and reduced prostate weight in males at 180 mg lasalocid/kg feed (5 mg/kg bw/day). Dogs appear to be more sensitive to the effects of lasalocid than rats.

The oral toxicity of lasalocid in chickens has been investigated in repeated dose studies. In subchronic dietary studies, dietary concentrations of up to 125 mg/kg feed (equivalent to approximately 7-11 mg/kg bw/day) produced no observable adverse effects. In a study in day-old chickens up to 9 weeks lasalocid sodium was administered *via* dietary admixture at concentrations from 75 to 375 mg/kg feed. No significant differences in mortality, body weight, feed efficiency, or haematology were seen up to

225 mg/kg feed. In a 13-week study in day-old chicks, increased mortality, reduced body weight gain, and reduced feed efficiency were observed at 225 and 375 mg/kg feed; no effects on haematology were observed. No adverse effects were observed at 75 mg lasalocid/kg feed or 150 mg lasalocid/kg feed (equivalent to approximately 8.8 mg/kg bw/day).

Reproductive toxicity, including developmental toxicity

Effects on reproduction

A single-generation reproduction study of lasalocid sodium was performed in rats. Males were dosed for 21 days prior to mating and during the 14-day mating period. Females were dosed for 21 days prior to mating, during the 14-day mating period, throughout gestation, and throughout lactation until lactation day 21. F0 body weights and food consumption were measured pre-test and at weekly intervals thereafter. Dams were also weighed at weekly intervals after the day of gestation. After delivery the litters were examined for external anomalies. Pups were counted, weighed and sexed at birth and weighed again at weekly intervals. On lactation day 21, pups were weaned and 60 animals/sex/dose were maintained for a 13-week toxicity study. There was no drug-related effect on clinical signs or mortality, in the F0 or F1 generations. Parental females treated with 10 mg/kg bw weighed significantly less than the control female group from day 0 of gestation onwards. Pup weight at birth was not affected by treatment. At lactation day 4, 7, and 14, weights of pups from the group of parent treated with 10 mg/kg bw were significantly reduced compared with control group pups. By lactation day 21, this difference was reduced and was no longer statistically significant. Abnormalities observed included gastroschisis (1 pup, at 1 mg/kg bw), abnormal flexion of the limbs accompanied by poor muscular coordination (1 pup, at 3 mg/kg bw), bilateral anophthalmia (1 pup, at 1 mg/kg bw) and 1 pup with multiple defects (10 mg/kg bw). There was no effect of treatment on the number of pregnancies or the percent pregnant, the length of gestation, the number of litters born, the average litter size, the average number of implantation sites per litter, the distribution by sex of viable pups, or the gestation, viability or lactation indices. The examination of foetuses was restricted to external observation. A NOEL for all effects of 3 mg/kg bw/day was established, based on the occurrence of reduced maternal weight gain at 10 mg/kg bw/day. A three-generation reproduction and teratology study was performed in rats, comprising the P1 generation and groups of 20 animals per sex of subsequent generations. Animals (male and female) received 0, 10, 35 and 120 mg/kg bw of lasalocid sodium with feed, during nine weeks prior to mating, continuing for three generations. In addition to the assessment of reproductive parameters, females from the first breeding of the first generation (P1F1a), sacrificed on gestation day 13, and the third mating of the third generation (P3F3c), sacrificed on gestation day 19, were used for teratological evaluation. Decreased bodyweight was seen in high-dose females during the growth phases in each generation, but was only statistically significant in P1 females. Slightly decreased body weight was also seen in P1 and P3 high-dose females during gestation, but was not statistically significant. Food consumption of high-dose P1 and P3 females was also slightly decreased during gestation, though this was only significant for P1 dams during the first week. Food consumption during growth phases was unaffected.

Pregnancy and fertility rates of high-dose groups were consistently decreased, though only significantly in the P3F3b. Weaning and lactation survival indices were also significantly decreased in the P3F3b high-dose offspring. Mean litter sizes at birth were reduced at 120 mg/kg feed in the 1st and 3rd generations. Pup body weights at birth were similar between groups, but at postnatal day four, P2 and P3 pups weighed less than controls. The number of corpora lutea and implantations were decreased in the mid- and high-dose groups in both the P1F1a and P3F3c generations. There was no effect on the incidence of visceral or skeletal abnormalities in the F3c generation, although an increase in delayed

ossification was seen at 120 mg/kg feed. No histopathological findings were seen in the F3b generation. The NOEL for this study was 10 mg lasalocid/kg feed (approximately 0.5 to 0.8 mg lasalocid/kg bw/day).

Developmental toxicity

A preliminary embryo-foetal study was performed in female New Zealand White rabbits; the objective of this was to find a dose range to be used in a later developmental oral toxicity study. In this study a dose-dependent decrease in bodyweight and food consumption at all levels of lasalocid sodium administered (1, 2 and 4 mg/kg bw/day) was observed. Treatment was administered daily from gestation days 6 through 28. Animals were examined daily for clinical signs and were checked twice daily for viability. Foetuses were examined for external abnormalities and the total weight of live foetuses per litter recorded. A dose-dependent decrease in bodyweight gain was seen. A decrease in the number of mean live implants, caused by an increase in the number of early and late embryonic deaths, was seen at 4 mg/kg bw. Foetal weight was decreased at all doses. One animal treated with 4 mg/kg bw had a shortened tail with a small skin flap. The data obtained from this preliminary study supported the inclusion of dose levels of 0, 0.5, 1, and 2 mg/kg bw/day of lasalocid sodium in the successive pivotal study for developmental toxicity performed in the rabbit. A NOEL from this study could not be identified. A second study was performed in female New Zealand White rabbits to detect the effects of lasalocid sodium in pregnant rabbits. The Dose levels were 0, 0.5, 1 and 2 mg/kg bw/day. This study was conducted according to GLP conditions. The animals were treated by oral route (gavage) from days 6 to 28 of gestation.

Animals were examined for clinical signs daily. Body weights and food consumption were recorded daily from day 4 or 5 of gestation. Animals were sacrificed and necropsied and foetuses examined on day 29 of gestation. No treatment-related maternal mortality was seen. Treatment of rabbits with lasalocid sodium was associated with a dose-related decrease in food consumption at 1 and 2 mg/kg bw. This led to a reduction in faecal output and significantly reduced body weight/weight gain at 2 mg/kg bw. These effects could be explained as related to the particular sensitivity of the gut flora in rabbits given certain antimicrobial agents. At 2 mg/kg there was a slight increase in the incidence of foetuses with corneal opacity. At 1 and 2 mg/kg bw/day there was a slight increase in the incidence of pale spleen. Only at 2 mg/kg bw/day the incidence of foetuses with jungals connected to maxilla was greater than control. The incidence of foetuses at 2 mg/kg bw/day with complete 13th supernumerary ribs and displaced pelvic girdle was marginally greater than the controls. At 2 mg/kg bw/day there was an increase in the incidence of foetuses with incomplete ossification. The NOEL for foetal toxicity was 0.5 mg lasalocid/kg/day (based on pregnancy performance and foetal body weight). The NOEL for maternotoxicity was considered to be 0.5 mg lasalocid/kg/day, based on systemic toxicity.

Genotoxicity

Lasalocid was tested in a series of *in vitro* mutagenicity test systems. In the rec-assay, Wilkins-Chalgren lasalocid was investigated for its potential for killing repair-deficient cells. Concentrations of 1, 10, and 100 µg/disc of lasalocid sodium, dissolved in dimethyl sulfoxide, were incubated with Bacillus subtilis M45 (DNA-recombination-deficient) and H17 (DNA recombination-proficient. After 24 hours incubation, the difference between the growth inhibitory zones for the two strains, for each concentration of lasalocid, was less than both the positive and negative controls. Hence, lasalocid sodium showed no DNA damaging effect in this assay. In the bacterial reverse mutation test assay, the mutagenic potential of lasalocid sodium was investigated, both in the presence and absence of metabolic activation, in *Salmonella typhimurium* strains TA1535, TA 1537, TA 98, and TA 100, and

Escherichia coli strains trp-B/r WP2 and trp-WP2 hcr -. The results of the bacterial reverse mutation assay indicate that lasalocid sodium, at concentrations up to 2000 µg/kg/plate, does not induce gene mutations in the strains tested, either in the presence or absence of metabolic activation. In the yeast assay, *Saccharomyces cerevisiae* D7 was used to detect the frequencies of gene conversion at the trp5 locus, mitotic recombination and other mitotic segregations using the ade 2 marker, and reverse mutations at the ilv 1-92 mutant allele. Lasalocid concentrations of 0, 0.05, 0.17, 0.50, 1.67, and 5 mg/ml were used, both in the presence and absence of metabolic activation. Lasalocid did not induce gene conversion, reverse mutation or mitotic crossing-over, either with or without metabolic activation. Lasalocid was assessed for mutagenic activity at the hypoxanthine guanine phosphoribosyl transferase (HGPRT) locus in Chinese hamster lung V79 cells. Cells were incubated with lasalocid at concentrations of 1 to 20 µg/ml in the absence of metabolic activation and 1 to 60 µg/ml in the presence of metabolic activation. Cytotoxicity was observed with high concentrations of lasalocid preventing assessment. The results of this study indicate that lasalocid does not induce gene mutation in the cultured mammalian cells used, either with or without metabolic activation.

Lasalocid was assessed for genotoxicity in the unscheduled DNA synthesis assay in primary cultures of male rat hepatocytes. Lasalocid concentrations of 0 to 12.5 µg/ml were tested initially, followed by concentrations of 0 to 5 µg/ml in a second assay. Lasalocid sodium was strongly cytotoxic with no viable cells available for assessment at concentrations of more than or equal to 4 µg/ml. Altered morphology occurred at 1 µg/ml. Lasalocid did not induce DNA damage resulting in repair, either in the presence or absence of activation, at concentrations up to those producing cytotoxicity. Therefore, no genotoxicity can be concluded from this assay. Lasalocid was assessed for its potential to induce chromosomal aberrations in human peripheral lymphocytes. It was tested three times in the absence of metabolic activation at concentrations of 0 to 8 µg/ml; concentrations of 0 to 10 µg/ml were also tested in the presence of metabolic activation. In each of the tests, cytotoxicity prevented assessment at the higher lasalocid concentrations. However, for lasalocid, at concentrations of 2 to 8 µg/ml, no clastogenicity were seen on this assay, either with or without metabolic activation.

It was concluded that lasalocid was not mutagenic.

Carcinogenicity

Two *in vivo* rodent carcinogenicity studies in mice and in rats have been reported. In a study in mice, lasalocid sodium was administered at concentrations of 0, 10, 35, and 120 mg/kg feed during two years, except during the first five weeks when low- and mid-dose concentrations of 20 and 60 mg/kg feed were used. No clinical signs or ophthalmic effects were observed, there was no effect on mortality, and no consistent effects on body weight or food consumption were seen. In males, and in females surviving until sacrifice, no treatment-related pathology or histopathology were observed and the incidence of neoplasms was similar across all groups. In females that died or were euthanised during the study, an increase in the incidence of lymphosarcoma was seen in the low- and high-dose groups (nine and ten cases, respectively, compared to three and five cases in the two control groups). However, no increase was seen either in decedents of the mid-dose group (four cases), or in the approximately 50% of animals from each treatment group that survived to the end of the study. No increased incidence of lymphosarcoma was detected in the treated males or terminal sacrificed females.

In the rat study, lasalocid sodium was administered with feed, at concentrations of 0, 10, 35, and 120 mg/kg feed. After one week, animals were mated. Treatment was continued throughout mating, gestation, and lactation. At weaning, F1 animals were selected for continued treatment for a further 130 weeks. Whilst survival in the study was low (21.8 to 43.6%) at study termination, survival rates at

week 104 were above 50% and the study is therefore considered adequate. Decreased serum blood urea nitrogen levels were observed in all treatment groups, including the low-dose group at weeks 26 and 78. No histopathological changes were noted in the kidneys. During the week 27 to 53 period, an increase in the number of mid- and/or high-dose females (higher incidence in high-dose) and high-dose males with slow grasping or righting reflexes was noted. There was no treatment-related pathology or histopathology and the incidence of neoplasms was similar across all groups. A conservative NOEL for all effects of 10 mg lasalocid/kg feed (equivalent to approximately 0.5 mg/kg/day in males and 0.6 mg/kg/day in females) was established, based on increased blood glucose and reduced urea-nitrogen concentrations, and increased adrenal weight at 35 mg/kg feed.

2.1.4. Studies on other effects including immunotoxicity and neurotoxicity

Lasalocid was evaluated for skin and ocular irritation in New Zealand rabbits. The studies suggest that lasalocid is non-irritating to the skin, but is an ocular irritant. The sensitisation potential of lasalocid has been investigated in a guinea pig maximisation test. Lasalocid-induced animals showed no greater response to challenge with lasalocid than animals induced with test vehicle only.

No specific studies on neurotoxicity have been provided. Published data showed that lasalocid induced histopathological changes in peripheral nerves in birds. Biophysical or biochemical changes in the nervous system were also described in dogs and rats. Acute and subchronic and chronic studies in dogs have demonstrated neuromuscular effects, evoked beginning with doses of 5 mg lasalocid/kg bw or above. At these dose levels, effects appeared to be transient and without histopathological findings. In one chronic study in rats an effect on grasping and righting was reported at several time points, at dietary doses corresponding to about 2 and 6 mg/kg bw.

In its previous evaluation the CVMP concluded that overall, the neurotoxicity effects were not considered to present a significant risk to the safety of consumers. However, the uncertainties and limited neurotoxicity data were taken into account in the calculation of the ADI by applying an increased uncertainty factor.

Further to re-consideration of the overall toxicity data and the EFSA conclusions the CVMP noted the following:

The potential for neurotoxic effects of lasalocid has been evaluated during repeated oral dose toxicity studies in the rat and the dog (the most sensitive species). In these studies detailed clinical signs, laboratory and histopathological changes of the brain (frontal and parietal cortex, cerebellum and pons), and peripheral nerves (sciatic nerve) and ophthalmic evaluations were provided.

In the three 13-week oral toxicity studies in rats, lasalocid doses of up to 20, 2 and 10 mg/kg bw/day, respectively, were used. There were no remarkable neurological effects observed in any of these studies performed in rats.

In one study of 2-year oral toxicity performed in rats no neurological effects were seen.

Two studies of thirteen week oral toxicity and 2-year oral toxicity were performed in dogs. Neurological examination included observations of posture, gait and reflexes.

In one of the 13-week studies, four dogs at the high dose group (10 mg/kg bw/day) developed short neurological changes consisting of a variable pattern of muscular weakness and/or tremors of the hind limbs, persisting for approximately 1 to 10 days (weeks 8 to 10). In one dog, these changes remained, to varying degrees, for 10 days and were accompanied by poor appetite, loss of body weight and a slight but statistically significant increase in alanine aminotransferase (ALT) to 10 mg/kg bw/day at week 4. During the neurological exam conducted after week 8, two male dogs receiving 10 mg/kg

bw/day developed temporary muscular weakness of the hind limbs, persisting 1 to 10 days. Two males displayed hind limb effects (awkward gait or bilateral tremor) however there were no observed effects at week 12 of examination. After 12 weeks, there were no findings on neurological examination that distinguished treated animals of the high-dose group from controls. At no time during the study were transient changes of the type noted in the high dose group in animals receiving 5 mg/kg/day or 2 mg/kg/day observed.

In the 2-year oral toxicity study in dogs intermittent paralysis of the limbs was observed for a single day during week 21 of the study in five of the high-dose group (180 mg/kg feed), however, the animals appeared normal within 24 hours of the observation and a reversion was not observed. One low dose female (10 mg/kg feed) exhibited moderate tremors for a single day during weeks 54 and 100 of the study. Gross and microscopic evaluations of animals at 6, 12 and 24 month necropsies revealed no abnormalities.

The signs of toxicity observed in these studies are consistent with the mode of action of ionophoric polyether ionophores; lasalocid directly translocates Ca^{++} because it forms complexes with divalent cations with a range of complexing and transport capabilities including primary amines, e.g., catecholamines producing excitation at the neuromuscular junction.

Lasalocid was well tolerated by dogs of both sexes when administered orally (by capsules) at doses of 2 or 5 mg/kg bw/day for 13 weeks. Neurological findings occur only at doses above 5 mg/kg bw/day.

In addition toxicological data in various species reported in the EFSA opinions indicate that lasalocid sodium is capable of inducing adverse neurological/neuromuscular effects if sufficiently high doses are used. These findings might be considered more as high-dose secondary pharmacological effects than signs of toxicity, as these are consistent with the mode of action of ionophoric polyether ionophores, and are comparable to the symptoms observed in the target animal species at levels exceeding the recommended dose.

The toxicological NOEL of 0.5 mg/kg/day from the 2-year chronic oral toxicity study in rat and the maternal toxicity study in rabbits is 10 times lower than the lowest dose resulting in neurological effects in dogs (5 mg/kg/day) and therefore the toxicological NOEL 0.5 mg/kg/day is considered adequately protective with regard to neurological effects.

EFSA previously considered that there was no reason to apply a higher safety factor than the standard 100 for the calculation of the ADI. Overall, taking into account the EFSA opinion and after reviewing the relevant data the CVMP agrees that the neurotoxicity has been sufficiently addressed and that there are no remaining concerns with regard to the limited data on neurotoxicity.

2.1.5. Calculation of the toxicological ADI or alternative limit

Previously the CVMP calculated a toxicological ADI of 2.5 µg/kg bw (i.e. 150 µg/person) based on the NOEL of 0.5 mg/kg/day from the 2-year chronic oral toxicity study in rat and the maternal toxicity study in rabbits and applying an uncertainty factor of 200 due to the limited data in respect of neurotoxicity.

Following review of the relevant data the CVMP considers that the initial concerns with respect to the sporadic neurotoxicological findings occurred at relatively high dose levels and were not seen at or near the NOEL of 0.5 mg/kg/day. The neurological effects induced by the higher doses tested in studies submitted can be considered as high-dose pharmacological secondary effects rather than toxicological effects *per se*.

Therefore the CVMP agreed that an uncertainty factor of 200 was not required and a standard uncertainty factor of 100 as retained in the EFSA evaluation could be applied in the calculation of the toxicological ADI for lasalocid.

Considering the NOEL of 0.5 mg/kg/day and applying an uncertainly factor of 100, the toxicological ADI of 5 µg/kg bw, i.e. 300 µg/person is established.

2.1.6. Overview of microbiological properties of residues

A reassessment of the microbiological data was provided taking into account the current VICH guidance; the evaluation previously carried out by the CVMP is summarised here below.

Disruption of the colonisation barrier

The Minimum Inhibitory Concentrations (MIC) of lasalocid sodium were determined against 15 strains of anaerobic bacteria representing the human intestinal microbiota. These comprised five isolates each of *Bacteroides spp*., *Fusobacterium spp*. and *Peptostreptococcus spp*. The test system was based on standardised agar dilution MIC methodology, as described in NCCLS guidelines. MIC determinations were performed using agar at pH 7.1, pH 6.0 and pH 8.5, conditions representing the pH range of the human intestinal ecosystem. For 14 of the 15 bacterial strains tested, the greatest difference between MIC results obtained at any two pH levels equated to two doubling dilutions in the MIC series. For a single *Fusobacterium* strain, lasalocid MIC obtained at pH 8.5 was three dilutions higher than that obtained at pH 7.1. However, the MIC obtained at pH 6.0 was also higher than that obtained at pH 7.1, by a factor of two dilutions. Thus, there was no clear trend with regard to variations in lasalocid MIC against this strain with agar pH.

A faecal binding study was conducted with lasalocid. The effect of faecal binding on the antimicrobial activity of lasalocid was studied by incubating selected lasalocid concentrations (0 to 100 µg/ml) with sterile pooled human faeces at concentrations of 0, 10, 20 and 50% w/v. After incubation of each combination for up to eight hours, faecal solids were removed by centrifugations and the supernatant liquid inoculated with a lasalocid-susceptible *Enterococcus faecalis* strain and incubated for 48 hours. Antibacterial activity in each inoculated preparation was determined after 24 hours and 48 hours by presence or absence of bacterial growth. Without prior incubation with faeces, a lasalocid concentration of 1 µg/ml consistently inhibited *E. faecalis* growth at each sampling point. Following brief incubation with 10% faeces, the initial lasalocid concentration required to inhibit *E. faecalis* growth increased to more than 100 µg/ml. This increase indicates that more than 99% of the lasalocid concentration was bound to faeces, reducing the concentration available in the supernatant. In the presence of all faecal concentrations, the degree of lasalocid binding remained consistent throughout the 8-hour incubation period and is regarded as irreversible. As there are presently no accepted and validated protocols for this type of investigation and due to concerns about the limit of detection of the analytical method, the number of strains tested, the presence of lasalocid on the supernatant, the lack of information about the binding of lasalocid and faeces in suspension and whether this binding is irreversible as well as the lack of positive controls, the retained fraction of the oral dose available for microorganisms was established as 0.1. The Minimum Inhibitory Concentrations (MIC) of sodium lasalocid against 84 bacterial strains representative of human gut microbiota were determined. Ten strains of *Bifidobacterium spp*., 10 strains of *Eubacterium spp*., 10 strains of *Clostridium spp*., 10 strains of *Peptostreptococcus spp*., 3 strains of *Lactobacillus acidophilus*, 9 strains of *Enterococcus spp*., 10 strains of *Streptococcus spp*., 3 strains of *Bacteroides fragilis*, 7 strains of *Fusobacterium spp*., 3 strains of *Escherichia coli*, 3 strains of *Proteus spp*., and 6 strains of *Salmonella enterica* serovar Enteritidis or serovar Typhimurium were tested. The methodology employed was MIC agar dilution as

described by the NCCLS for anaerobic bacteria (M11-43). There were differences between MIC50 for the different media that could be explained by binding of lasalocid to proteins, MIC50 on Wilkins-Chalgren media would be considered the most appropriate for the assessment of the Minimal Inhibitory Concentration of sodium lasalocid. In an additional study the MIC of sodium lasalocid against 30 bacterial strains, representing the normal human intestinal microbiota, was determined. Ten isolates each of *Bacteroides spp*., *Fusobacterium spp*. and *Peptostreptococcus spp*. were sourced from the faecal microbiota of healthy unmedicated humans. The test system was standardized agar dilution MIC methodology as described for NCCLS.

Increase of the population of resistant bacteria

Three *in vitro* studies were performed. Three sensitive bacterial strains (*Staphylococcus aureus*, *Enterococcus faecalis*, and *Clostridium perfringens*) were serially cultured 20 times in the presence and absence of sub-lethal concentrations of lasalocid. The potential of cross resistance was also investigated. A non-significant selective resistant pressure in the presence of lasalocid was observed. No cross resistant selection, on a panel of eight antimicrobials, was observed. Lasalocid sodium does not present an inhibitory effect on gram-negative bacteria however has good activity against the gram-positive bacteria tested. The addition of lasalocid to animal feed is unlikely to promote increased antibiotic resistance among the animal gut microflora.

2.1.7. Calculation of the microbiological ADI

For the determination of the microbiological ADI the CVMP previously considered, the MIC data for *Fusobacterium spp*., *Escherichia coli*, *Proteus* spp., and *Salmonella enterica* were excluded due to lack of sensitivity and the values for *Bifidobacterium* spp*., Eubacterium* spp*., Clostridium* spp*., Peptostreptococcus* spp*., Lactobacillus acidophilus, Enterococcus* spp*., Streptococcus* spp*.,* and *Bacteroides fragilis* from both studies were used to determine the overall MIC_{50}. The lower 10% confidence limit ($CL10\%_{lower}$) of MIC_{50} was 0.134 µg/ml.

The microbiological ADI previously calculated by the CVMP was 4.91 µg/kg/day bw (i.e. 294 µg/person).

Since the previous evaluation the VICH GL 36 on Studies to Evaluate the Safety of Residues of Veterinary Drugs in Human Food: General Approach to Establish a Microbiological ADI, entered into force. The previously submitted data were reassessed according to the new VICH guideline and are summarised in the table below.

	Resistance criteria	
Organism (source)	**MIC**	**Log2(MIC50)-Log2(minMIC50/2)**
Escherichia coli (McConville, 1998)	R*	R*
Enterococcus (McConville, 1998)	0.5	4
Bacteroides (Pridmore, 2004a)	32	10
Fusobacterium (Pridmore, 2004a)	1	5
Bifidobacterium (McConville, 1998)	0,25	3

Eubacterium (McConville, 1998)	0.125	2
Clostridium (McConville, 1998)	0.125	2
Peptococcus/ Peptostreptococcus (Pridmore, 2004a)	2	6
Streptococcus (McConville, 1998)	0.0625	1
Lactobacillus (McConville, 1998)	0.125	0.2
Proteus/Salmonella	R*	R*
MEAN	3.88889	
STDEV	2.80377	
T(0.10, df)	1.39682	
Lowe 90% CI	2.58344	
MIC (calc)	**0.1873**	

R*: organism not used in calculation, due to inherent resistance.

The equation for calculation of the microbiological ADI for the disruption of the colonisation barrier as per VICH GL 36 is reproduced below:

$$ADI = \frac{MIC_{calc} \times \text{Mass of Colon Content (220 g/day)}}{\text{Fraction of oral dose available to microorganisms} \times \text{60 kg person}}$$

Where:

- MIC $_{CALC}$: derived from the lower 90% confidence limit for the mean MIC_{50} of the relevant genera for which for which the drug is active.
- Fraction of the oral dose: 0.1 (as concluded by the CVMP in its previous evaluation following the assessment of the effect of faecal binding on the antimicrobial activity of lasalocid).

The microbiological ADI for lasalocid with regard to the disruption of the colonisation barrier has been determined using the formula above as follows:

$$ADI = \frac{0.1873 \times 220}{0.1 \times 60} = 6.8 \text{ µg/kg bw i.e. } 412 \text{ µg/person}$$

With regard to the increase of the population of resistant bacteria the CVMP considered that although lasalocid sodium had a good activity against the gram-positive bacteria tested, as the resistance selection pressure in the presence of lasalocid was not significant, no cross resistant selection was observed. Additionally, as lasalocid sodium does not exert an inhibitory effect on gram-negative bacteria, the addition of lasalocid to animal feed is unlikely to promote a significant increase of antibiotic resistance among the animal gut microflora and therefore a specific microbiological ADI to consider development of resistance was not deemed necessary. Therefore the ADI calculated in relation to the disruption of the colonisation barrier is considered the relevant microbiological ADI for lasalocid (6.8 µg/kg bw i.e. 412 µg/person).

2.1.8. Observations in humans

No data on observations in humans are available. Lasalocid sodium is not used in human medicine and was therefore not classified as a critically important antibiotic for humans by the WHO list of critically important antimicrobials for human medicine (2011, 3rd edition).

2.1.9. Findings of EU or international scientific bodies

The EFSA Scientific Panel on Additives and Products or Substances used in animal feed also assessed a product that contains lasalocid sodium further to a request from the European Commission to re-evaluate the substance and give advice on its efficacy and safety. An ADI of 5 µg/kg bw/day was established based on the NOEL of 0.5 mg/kg bw/day derived from a 2-year chronic toxicity study in rats and a maternal toxicity study in rabbits applying a safety factor of 100.

2.1.10. Overall conclusions on the ADI

The toxicological ADI of 5 µg/kg (300 µg/person) was calculated based on the NOEL of 0.5 mg/kg bw from the 2-year chronic oral toxicity study in rat and the maternal toxicity study in rabbits and applying an uncertainty factor of 100. The microbiological ADI was re-calculated in accordance with VICH GL 36 to be 412 µg/person which is higher than the toxicological ADI.

The toxicological ADI of 5 µg/kg i.e. 300 µg/person is therefore considered the relevant ADI for assessing the risk for the consumer and established as the overall ADI for lasalocid.

2.2. Residues assessment

2.2.1. Pharmacokinetics in target species

The evaluation previously carried out by the CVMP with regard to pharmacokinetics is summarised here below.

The pharmacokinetics of ^{14}C-labelled lasalocid was studied in chickens after 16 days administration of 75 mg/kg of unlabelled lasalocid in feed followed by a 3-day administration of capsules containing labelled lasalocid at a dose rate of 5 mg/kg bw. Peak plasma concentrations of labelled lasalocid were 5.62 µg/ml and were obtained 2 hours after the last capsule administration. The lasalocid blood elimination half-life was 3 and 48 hours after the last administration the lasalocid concentrations were reduced to 0.39 µg/ml.

After 7-day administration of capsules containing the equivalent of 125 mg/kg bw of ^{14}C-lasalocid in feed to 25 one day old chickens, an average concentration of 0.56 µg/ml of radioactive residues was found in plasma. The plasma radioactive concentration decreased to 0.003 µg/ml 7days after treatment. Eighty-eight percent of the administered labelled lasalocid was eliminated in the excreta at 0 days after administration, the dose fraction excreted 7 days after the end of the treatment was 91%. After the analysis of the contents of lasalocid A in the excreta of the animals 7 days after administration, 74.3% to 76.9% of the administered dose was recovered in the excreta as lasalocid A, 0.8 to 4.1% as lasalocid A homologues, and 0.3 to 4.4% as unidentified components.

Lasalocid is metabolised in a similar metabolic pathway in both target food animal species (chickens and turkeys) and rats. A number of unidentified fractions were present. The radioactivity in the liver fractions are quite similar between chickens, turkeys and rats. Unchanged lasalocid represents in the

total liver and faeces 3.8% and 10.0% for turkeys, 11.4% and 12.0% for chickens and 31.9% and 43.7% for rats, respectively. Chickens and turkeys contained similar metabolites in faeces compared to those found in the monogastric mammalian species (dog, swine and rat).

2.2.2. Residue depletion studies

The evaluation previously carried out by the CVMP with regard to residue depletion studies is summarised here below.

The residues of ^{14}C-labelled lasalocid were studied in chickens after 16 days of administration of 75 mg/kg of unlabelled lasalocid in feed followed by a 3 days administration of capsules containing labelled lasalocid at a dose rate of 5 mg/day. After analysis of the radioactive contents of edible tissues, the peak concentrations of radioactive residues were 10300, 760, 1400 and 3000 µg/kg in liver, muscle, fat and kidney respectively, and they were obtained 2 hours post treatment suppression. After 7-day administration of 127 mg/kg feed ^{14}C-labelled lasalocid to broiler chickens, the total radioactive residue concentration was compared to the lasalocid A concentration. Eight hours after treatment, the radioactive concentration found in liver was 2.01 µg/ml while the concentration of lasalocid A was 0.094 µg/ml. The lasalocid A in liver represents 4.6% of the radioactive residues.

Thirty Cornish Cross chickens were treated with 125 mg/kg feed of unlabelled lasalocid for 34 days in feed followed by 21 days of treatment with 132 mg/kg feed of radiolabelled lasalocid. Groups of animals were sacrificed at 0, 1, 2, 3, 4 and 5 days after treatment suppression and edible tissues were collected. The radioactive tissue residues were quantified. At 0 days after treatment the fat, skin, kidney, muscle and liver radioactive concentrations were, respectively, 860, 1590, 2480, 610 and 11 930 µg/kg. Twenty four hours after the treatment the fat, skin, kidney, muscle and liver radioactive concentrations were, respectively, 140, 220, 360, 60, and 2630 µg/kg. Forty-eight hours after treatment the fat, skin, kidney, muscle and liver concentrations were, respectively, 60, 130, 230, 30, and 1720 µg/kg. The radioactive residue concentration in skin, fat, muscle and kidney were below 200 µg/kg at 3 to 5 day after treatment. The radioactive concentrations in liver at 3, 4 and 5 days post-treatment were, 1590, 1370 and 1150 µg/kg respectively, indicating that this tissue shows a relatively slow elimination pattern of the residues.

Unlabelled lasalocid at a concentration of 90 mg/kg feed was administrated to 40 broiler chickens during 14 days. Blood, liver and muscle lasalocid concentrations were quantified, using an ELISA technique, 0 to 7 days after treatment. The elimination half life of lasalocid was 11, 36, and 41 hours for serum, liver and muscle, respectively. Seven days after treatment, only liver showed lasalocid concentrations above 10 µg/kg.

After 7-day administration of capsules containing the equivalent of 125 mg/kg of ^{14}C-lasalocid in feed to 25-day broiler chickens, the total radioactive concentrations in edible tissues were quantified. Also, the lasalocid A concentrations were quantified using an HPLC technique. After analysing the total tissue residues, some lasalocid A homologues were found and up to seven unknown metabolites were detected. At 0 h withdrawal time, the total radioactive residues were 1.22, 0.40, 0.08 and 0.43 µg/g in liver, kidney, muscle and skin+fat, respectively. The lasalocid A concentrations at the same time were 0.24, 0.122, 0.04 and 0.21 µg/g for liver, kidney, muscle and skin+fat, respectively. The ratios for lasalocid A concentrations to total radioactive concentration, at 0 h withdrawal time, were 0.22, 0.41, 0.55, and 0.52 for liver, kidney, muscle and skin and fat, respectively. At 24 hours after administration, the marker to total residue ratios were 0.086, 0.142 and 0.283 for liver, kidney and skin and fat, respectively. Lasalocid A was confirmed as the marker residue. At 0 hours and 24 hours withdrawal period the total radioactive residue consumption, taking into account the food basket proportions, represents 126% and 68% of the ADI, respectively.

The tissue residues of lasalocid in pheasants and quail were studied. After administration of 90 mg/kg of lasalocid for 27 days to quails, the tissue concentrations (skin, muscle and skin + fat) were analysed. The highest tissue concentrations were found in skin (298.3 µg/kg, 55 µg/kg, 30.8 µg/kg and 33.7 µg/kg at 0, 3, 6 and 9 days after treatment, respectively) and they were ten fold higher than the lasalocid muscle concentrations. After 7 days administration of medicated feed with 132 mg/kg of lasalocid sodium to pheasants, the liver and skin and fat lasalocid A concentrations were 28.5 and 30.7, respectively. Lasalocid A, as marker residue, was found in quail and pheasant tissues. The studies provided were according to the requirements for extrapolation to poultry of the Notes for Guidance on Risk Analysis Approach for Residues of Veterinary Medicinal Products in Food of Animal Origin (EMEA/CVMP/187/00- FINAL) and on the Establishment of Maximum Residue Limits for Minor Animal Species (EMEA/CVMP/153a/97-FINAL)

Data from newly performed residue studies to determine the residues of lasalocid A in tissues of broiler chickens were provided.

In a new residue depletion study in all edible tissues from chickens for fattening 48 healthy Ross broiler chickens (24 male, 24 female) were treated with feed containing lasalocid sodium at a dose level of 130 mg/kg up to 42 days of age. Samples of edible tissues liver, kidney, muscle (breast) and skin and fat were collected at sacrifice times. Lasalocid A concentrations in tissues were determined using a validated LC-MS/MS method with electrospray ionization. The limit of quantification (LOQ) for this method was
5.0 µg/kg for all tissues.

At 0 day withdrawal (on-feed) the average tissue concentrations of lasalocid A were above the current MRL values established in the European Union (i.e. 20 µg/kg for muscle, 100 µg/kg for skin and fat and liver, and 50 µg/kg for kidney). Within one day after administration, the lasalocid A concentrations were rapidly depleted, but were still over the MRL values in all edible tissues. By the second day after administration, tissue concentrations were either below the MRL (skin and fat) or approaching MRLs (liver, kidney and muscle). Within three days after administration the average concentrations in all edible tissues were below the corresponding MRLs, although samples of liver (3 out of 12) were over the MRL values after three days withdrawal. Results of the stability analysis indicated that the concentration was -5.4% of the initial concentration, demonstrating good stability in the supplemented feed. The applicant has proposed the following new MRL values: liver and skin and fat 300 µg/kg, kidney 150 µg/kg and muscle 60 µg/kg. Taking the results of this study, the mean lasalocid A residues were below these MRLs in liver, and kidney, and skin and fat at 1 and 2 days after treatment in liver and kidney and skin and fat respectively. Muscle residues were less than the MRL value at 3 days.

In a newly provided study of residue depletion in muscle and skin and fat obtained from broiler chickens treated with lasalocid medicated feed at 113 g/ton for 42 days followed by treatment with non-medicated feed for up to 10 days skin and fat and breast muscle samples were collected from each bird and lasalocid A was analysed using validated LC/MS/MS methods with a LOQ of 1 µg/kg. Lasalocid A residues were depleted rapidly in an *alpha depletion phase* between 3 and 24 hours withdrawal time in both skin and fat and muscle but residue depletion in the *beta depletion phase* was much slower with lasalocid still detectable at 240 hours after treatment in both tissues. All skin and fat samples were below the established MRL (100 µg/kg) and the proposed revised MRL (300 µg/kg) by 24 hours after treatment. All muscle samples were below the established MRL (20 µg/kg) by 24 hours, but due to being on the plateau of the *beta depletion phase*, one sample at 120 hours after treatment and one at 168 hours after treatment approached the MRL value. Comparing the results to the newly proposed muscle MRL value (i.e. 60 µg/kg) all of the muscle samples would be well below this MRL level by 24 hours after treatment.

A study to investigate the residue depletion of lasalocid in growing turkeys following administration of lasalocid in the diet for 112 consecutive days was provided. Groups of 6 birds (3 male and 3 female) were treated with feed containing 130 mg/kg lasalocid sodium followed by sacrifice of the birds and the collection of edible tissues at 0, 72, 120, 168 and 240 hours after treatment. Lasalocid A in tissues was analysed using a validated LC/MS/MS method (limit of quantification: 50 µg/kg for liver and skin and fat, 25 µg/kg for kidney and 10 µg/kg for muscle).

Lasalocid A residues at 0 hours attained the highest levels in skin and fat and liver, followed by kidney with much lower levels found in muscle. The concentrations depleted rapidly by the next slaughter time point, with all residues below the limit of quantification by 72 hours after treatment. Residues of lasalocid in liver, kidney and muscle were all below the LOQ at 120, 168 and 240 hours after treatment. Residues of lasalocid A in skin and fat were seen above the LOQ (106 and 59.4 µg/kg) in two samples at 120 hours after treatment. All skin and fat samples at 168 and 240 hours were below the LOQ.

The effect of feeding lasalocid sodium on edible tissue residues in farmed quail was studied. There was no data from kidney and liver. The rate of depletion of the skin and fat residues appears to be slower than that found in a chicken study. In this case the residue was lower than the LOD (10 µg/kg) after 2 to 3 days after treatment. The quail skin residue remained at 30 µg/kg at 6 and 9 days after treatment. The rate of depletion of the muscle residue may be similar to that of the chicken and reached a level lower than the LOQ within 2 to 3 days.

Residue depletion of lasalocid sodium in pheasants was studied. The analytical method was HPLC with fluorimetric detection which was validated at concentrations in the range of 20 to 500 µg/kg for all tissues. Residue concentration of lasalocid A in liver, kidney, muscle and skin and fat were lower than the MRL values (100, 50, 20 and 100 µg/kg, respectively) in 5 out of 6 birds 24 hours after treatment indicating a rapid depletion. Within 120 hours after treatment, all tissues in all birds were below the corresponding tissue MRL values. One bird at 168 hours after treatment contained residues in skin and fat higher (413 µg/kg) than the corresponding MRL value (100 µg/kg), but all the remaining tissues were less than the corresponding MRL values. Based on the results of this study the presence of lasalocid A (marker residue) in edible tissue from pheasants can be confirmed.

Selection of marker residue and ratio of marker to total residues

Lasalocid A was previously retained as the marker residue in liver, kidney, muscle and skin and fat. This fact is related to the metabolic pathway, which indicates that lasalocid A was the marker residue in all tissues of each sex.

The ratios of marker to total residues calculated at zero days after treatment are 0.55 in muscle, 0.52 in skin and fat, 0.22 in liver, 0.4 in kidney, and 0.37 in eggs and were retained from the previous CVMP evaluation.

2.2.3. Monitoring or exposure data

No monitoring or exposure data were provided.

2.2.4. Analytical method for monitoring of residues

Several LC/MS/MS methods with limits of quantification in the range of 1 to 5 µg/kg were presented for monitoring of residues in chicken tissues. However, only one of the methods was validated for all the relevant tissues. The limit of detection for lasalocid A was determined to be 0.6, 0.56, 0.70, and

0.67 µg/kg for broiler chicken muscle, liver, kidney and skin and fat respectively and the limit of quantification was 5 µg/kg for all tissues examined.

The analytical method was validated in accordance with the requirements of Volume 8 of the Rules for Veterinary Medicinal Product in the European Union and is considered acceptable for residue monitoring purposes.

The relevant European Reference laboratory has reviewed the proposed analytical method and is in agreement with the above conclusion.

An additional LC/MS/MS method for monitoring of residues in turkeys tissues was also provided.

2.2.5. Findings of EU or international scientific bodies

The EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed also assessed a product containing lasalocid sodium for use as a feed additive in poultry further to a request from the European Commission to re-evaluate the substance and give advice on its efficacy and safety.

The MRLs established with regard to residues of veterinary medicinal products under Regulation 37/2010 are also applicable to the authorisation of the substance as a feed additive.

In September 2007 the EFSA produced an opinion on cross-contamination of non-target feedingstuffs by lasalocid authorised for use as a feed additive, the opinion concludes that adverse health effects in consumers resulting from exposure to lasalocid residues in products from animals exposed to feed cross-contaminated even up to a level of 10%, are unlikely.

3. Risk management considerations

3.1. Potential effects on the microorganisms used for industrial food processing.

Although lasalocid has microbiological properties, considering that food products derived from poultry are not used in industrial food processing, in which microorganisms are used, no data on the effects of the substance on industrial food processing are required.

3.2. Other relevant risk management considerations for the establishment of maximum residue limits.

None.

3.3. Elaboration of MRLs

Based on the residue depletion data, distribution of marker residues between target tissues and ratios of marker to total residues and taking into account the toxicological ADI of 300 µg/person, increased MRL values for muscle, liver, kidney and skin and fat of poultry can be calculated.

Using mean marker to total residue ratios determined at 0 hours withdrawal time of 0.22 in liver, 0.41 in kidney, 0.55 in muscle and 0.52 in skin and fat, increased MRL values can be recommended as follows:

- Muscle: 60 µg/kg
- Skin and fat in natural proportions: 300 µg/kg
- Liver: 300 µg/kg
- Kidney: 150 µg/kg

Calculation of theoretical daily intake of residues

Edible tissue or products	Daily consumption (kg)	MRL proposal µg/kg)	Ratio of the marker/total residue	Amount per edible tissue or product
Muscle	0.30	60	0.55	32.72
Skin/Fat Poultry	0.09##	300	0.52	51.92
Liver	0.10	300	0.22	136.36
Kidney Poultry	0.01	150	0.41	3.66
Eggs	0.10	150	0.38	40.54
Estimated total daily intake (µg/person)				**265.2**

fat and skin in natural proportion

Based on the new recommended MRLs the theoretical maximum daily intake (TMDI) of residues corresponds to 265.2 µg of lasalocid which represents approximately 88.4 % of the ADI.

3.4. Considerations on possible extrapolation of MRLs

In line with Article 5 of Regulation (EU) No 470/2009 the CVMP considered the possibility of extrapolating the maximum residue limits established for lasalocid to other food producing species and food commodities. Taking into account the current scientific knowledge the recommendations on extrapolation are justified as follows:

Animal species/ food commodities	**Extrapolation possible (Yes/No)**	**Justification**
Sheep	No	Existing data indicate that the pattern of metabolites seen in rats, dogs, cattle and poultry is similar. Based on this existing inter-species metabolism data, and that cattle and sheep are related species (ruminants) the assumption could be made that lasalocid A would be the predominant residue in sheep and so would be a suitable marker residue. However, no specific pharmacokinetic or residue data were available for sheep and therefore the assumption related to the marker residue could not be confirmed and the ratio of marker to total residues could not be derived. Sheep meat is consumed on a regular basis and in large quantities. Species specific data are therefore considered necessary to allow adequate evaluation of the risk to consumer

		safety posed by residues in sheep tissues. No analytical method for monitoring of residues in sheep tissues was available for evaluation.
Goats (including milk)	No	No pharmacokinetic or residue depletion data were available for goats. Species specific metabolism and residue data are needed in order to draw conclusions on safe MRL levels. No data are available to demonstrate that the analytical method for monitoring of residues is applicable for monitoring of residues in goat tissues or milk.
Pigs	No	No pharmacokinetic or residue depletion data were available for pigs. As pig meat is consumed on a regular basis and in large quantities across the EU, species specific data are considered necessary in order to quantify the possible impact on consumer safety of exposure to residues. No analytical method for monitoring of residues in pig tissues was available for evaluation.
Horses	No	Existing data indicate that the pattern of metabolites seen in rats, dogs, cattle and poultry is similar and it can be expected that lasalocid A would be a suitable marker residue in horses. However, no data are available to demonstrate that the analytical method used for monitoring of residues in cattle and poultry is applicable for monitoring of residues in horse tissues.
Rabbits	No	Existing data indicate that the pattern of metabolites seen in rats, dogs, cattle and poultry is similar and it can be expected that lasalocid A would be a suitable marker residue in rabbits. However, no data are available to demonstrate that the analytical method used for monitoring of residues in cattle and poultry is applicable for monitoring of residues in rabbit tissues.
Fin fish	No	Metabolism is generally less complicated in fish than in cattle and poultry. Consequently, if the marker residue is lasalocid A in cattle and poultry it can be assumed that lasalocid A would also be a suitable marker residue in fish meat. However, no analytical method for monitoring of residues in fish meat was available for evaluation.
Milk	No	No data are available that would allow conclusions to be drawn on the appropriate marker residue or marker to total residues ratio to use in milk. Milk is consumed on a regular basis and in large quantities and consequently data on residues in this commodity are considered necessary in order to allow adequate evaluation of the risk to consumer safety posed by residues in milk. No analytical method for monitoring of residues in milk was available for evaluation.

Honey	No	Residue depletion in honey does not occur through metabolism and consequently conclusions drawn from data in other food products cannot be extrapolated to honey. Honey specific data are required in order to allow adequate evaluation of the risk to consumer safety posed by residues in honey. No data are available to demonstrate that the analytical method used for monitoring of residues in cattle and poultry tissues is applicable for monitoring of residues in honey.

3.5. Conclusions and recommendation for the establishment of maximum residue limits

Having considered that:

- the toxicological ADI of 5 µg/kg bw (i.e. 300 µg/person) was established as the overall ADI for lasalocid;
- lasalocid A was retained as the marker residue;
- the ratios of marker to total residues calculated at 0 days were 0.55 in muscle, 0.52 in skin and fat, 0.22 in liver, 0.41 in kidney, and 0.37 in eggs;
- residues concentrations were persistently low in muscle and fat and both target tissues were chosen for monitoring of residues;
- a validated analytical method for the monitoring of residues of lasalocid in edible poultry tissues (liver, kidney, muscle and skin and fat) is available;

the Committee recommends the modification of maximum residue limits for lasalocid in poultry in accordance with the following table:

Pharmaco-logically active substance	Marker residue	Animal species	MRLs	Target tissues	Other provisions	Therapeutic classification
Lasalocid	Lasalocid A	Poultry	60 µg/kg 300 µg/kg 300 µg/kg 150 µg/kg 150 µg/kg	Muscle Skin and fat in natural proportions Liver Kidney Eggs	NO ENTRY	Anti-infectious agents/ Antibiotics

4. Background information on the procedure

Submission of the dossier 23 October 2012

Steps taken for assessment of the substance

Application validated: 7 November 2012

Clock started: 8 November 2012

List of questions adopted: 11 April 2013

Consolidated response to list of questions submitted:	13 September 2013
Clock re-started:	14 September 2013
List of outstanding issues adopted:	9 October 2013
Clock re-started:	14 November 2013
CVMP opinion adopted:	12 December 2013